TILLINA MAGNA: MICRONUCLEAR NUMBER, ENCYSTMENT AND VITALITY IN DIVERSE CLONES; CAPABILITIES OF AMICRONUCLEATE RACES

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It is well established that the number of micronuclei in *Tillina magna* is highly variable. For example, Gregory (1909) found 6–10, and Ilowaisky (1921), in a ciliate which he called *Pseudocolpoda cochlearis cienkowskii,* reported 2–6. An examination of Ilowaisky's text and figures shows conclusively that his ciliate was *T. magna* Gruber (1879) as Kahl (1931, p. 282) pointed out. Kahl apparently regarded six as the typical number. Bresslau (1922) observed the nuclei in sufficient detail to note the extrusion of macronuclear material at division and the presence of several micronuclei, though he reported no counts of their actual number. The writer (1946), in a study dealing chiefly with the history of the nuclei during division and encystment, counted the number of micronuclei in 100 individuals (50 active and 50 encysted) of each of three clones, and found that it varied from 6 to 11 in one clone and from 4 to 6 in the other two. Thus the number was found to vary in different individuals of the same clone, and the mean number was found to vary in different clones. Active specimens and resting cysts of any particular clone had on the average like numbers of micronuclei. Contrary to statements in the literature, it was shown that the micronuclei divide at the time of cell division, and not indiscriminately or without regard to cell division. The mechanism by which two daughter cells may receive unlike numbers of micronuclei at division, thus accounting for variations in number within a clone, was described.

The significance of the wide variation in micronuclear number is unexplained. Structurally and physiologically an individual having only 4 micronuclei does not appear to be fundamentally unlike one having 11 micronuclei. The same condition prevails in the closely related species, *T. canalifera,* which I was formerly disposed to regard (1945) as identical with *T. magna*. However, on the basis of information furnished me by Dr. George W. Kidder, of Amherst College, it appears that *T. canalifera* merits recognition as a valid species, chiefly because of the very conspicuous nature of its canal system. In *T. canalifera,* Turner (1937) reported 4–14 micronuclei, though Burt, Kidder, and Claff (1941), in specimens obtained from the late Dr. Turner, found only one. Hence, it is clear that the micronuclear number may vary from 1 to 14, yet the evidence indicates that the uninucleate and multinucleate races were equally cultivable, vigorous, and capable of producing normal resting cysts.

The present study of *T. magna* was undertaken in order to obtain additional information concerning two points: (1) the normal variation in micronuclear number in various natural races and (2) the significance of such variation. The investigation of the first point is readily feasible, in that the micronuclei may be counted with absolute certainty in Feulgen preparations of favorably oriented resting cysts or medium-sized trophic specimens. The investigation of the second point, though less suited to direct approach, is not impracticable. A number of questions arise, some of which submit to experimental analysis. For example, is the number of micronuclei related in any way to size, whether of trophic specimens, division cysts or resting cysts; to division rate; to vitality, meaning capacity to endure with undiminished vigor as generations pass; to ability to produce resting cysts; to the viability of such cysts; or to ability to excyst? Of the foregoing measurable characters, the following were selected as being most readily amenable to experimental investigation: ability to produce resting cysts, size and viability of such cysts, capacity to excyst, division rate and vitality. These then, will be considered in relation to micronuclear number, though not all of them will receive equal consideration. The study assumed unlooked-for interest when it became evident that three of the races were amicronucleate. Thus a comparison of the potentialities of micronucleate and amicronucleate clones became possibile.

Materials and Methods

Twenty clones of *T. magna,* to be designated numerically, were used in the study. The progenitors of these respective clones were collected in a meadow, known locally as Sparrow's Pasture, in the vicinity of Chapel Hill, North Carolina. Comparisons with clones from other sources were desirable, but unfortunately attempts to collect *Tillina* elsewhere in the Chapel Hill region, and in the vicinity of Stanford University, California, and Woods Hole, Massachusetts, were unavailing. In this study a clone refers to all the progeny which were derived asexually from a single resting cyst or trophic specimen. The intervention of encystment and subsequent excystment is not considered to be a valid reason for changing the clonal designation, since there is no evidence that encystment in *Tillina* involves a sexual process which might change the genetic constitution of the clone. (It should perhaps be recalled that *Tillina,* like its near relative *Colpoda,* reproduces within a thin-walled temporary cyst, from which usually four progeny emerge shortly as a result of two successive divisions. The term encystment, as used in this study, does not refer to these temporary division cysts, but to the protective or resting cysts.) It is not definitely established that all of the twenty clones were genetically different, since their histories prior to their period of laboratory life were unknown.

The progenitors of clones 1, 2, 6, 8, 9, 11, 12, 13, 15, 17, 18, and 19 were taken as active specimens on Sept. 10, 1945, and these clones were therefore cultured simultaneously in the early part of the study. Eight of the foregoing clones, namely, 6, 11, 13, 18, 15, 19, 1, and 17, have already been reported on briefly under the numerical designations 1 to 8, respectively (Beers, 1946a). In the present paper my original numerical designations of all clones have been changed for the convenience of the reader in using the accompanying tables. The progenitors of clones 3, 14, 16, and 20 were isolated on Feb. 4, 1946, when dried leaves and debris, after 8 months of storage at 19° C., were immersed in weak hay infusion; these clones were therefore cultured simultaneously. It is evident that they were derived from dried cysts. The progenitors of clones 4, 5, 7, and 10 were isolated on April 8, 1946, when moist leaves and debris, which had recently washed against the bases of willow saplings in the meadow, were immersed in hay infusion; these clones were maintained in culture simultaneously toward the end of the study. They were undoubtedly derived from wet cysts.

An attempt was made to maintain each of the clones in pure-line culture for a period of 60 days. Sixteen of the clones were readily cultivable and continued with undiminished vigor throughout the period; four were intractable in that their division rates declined and the lines encysted well before the end of the period. Thus the laboratory histories of the clones varied, although the conditions of culture were uniform. The details, in relation to micronuclear number, will follow.

Each clone was cultured in depression slides in the form of four sub-lines. These were maintained at 23° C. in 0.05 per cent lettuce infusion to which suitable quantities of *Pseudomonas fluorescens,* grown on nutrient agar, were added as food. Previous experience has shown that this general procedure, combined with daily isolations and transfers to fresh environments, meets adequately the cultural needs of *Tillina* (Beers, 1944, 1945). Records were made daily of fission rates and other points of interest.

Surplus animals from the lines were stained on cover glasses by the Feulgen method in order to make micronuclear counts of active specimens. Small stock cultures of each clone furnished precystic specimens when the food supply neared depletion. These specimens were removed and allowed to encyst on cover glasses in the manner described by Beers (1946). Thus convenient preparations were available, first for making measurements of living cysts, and then for Feulgen staining. All measurements and micronuclear counts of cysts were made on *single* resting cysts. These are the common type. They are practically spherical and therefore well suited for making accurate measurements.

NORMAL VARIATION IN NUMBER OF MICRONUCLEI

The data bearing on diversity in micronuclear number in the twenty clones are summarized in Table I, in which the clones are arranged and numbered in the order of decreasing mean numbers of micronuclei. The data, ignoring for the present the mean diameters of resting cysts, are largely self-explanatory. A few points deserve special mention.

In any particular clone both active specimens and resting cysts showed practically the same extremes of variation (range) in micronuclear number and had essentially the same mean number of micronuclei.

In different clones the mean numbers of micronuclei were extremely variable. Some clones (e.g., 1 and 2) had consistently high mean numbers; others (e.g., 16 and 17), consistently low numbers, with many intergrades between these extremes.

Clones 18, 19, and 20 were amicronucleate. This statement is not based on casual observation, but on an intensive study of these clones. In trophic specimens and resting cysts the micronuclei of *Tillina* are not disposed toward secretiveness. They are never imbedded in the macronucleus. Each has an endosome which stains intensely and conspicuously by the Feulgen method. In mature resting cysts only the rod-shaped or ellipsoid macronucleus and the micronuclei stain to any appreciable extent; there is nothing in the cytoplasm to conceal the micronuclei. In trophic specimens it is true that the food vacuoles also stain, but the micronuclei always lie in the clear peri-macronuclear space and are not in a position to be concealed by the vacuoles. Moreover, considerable numbers of individuals of clones

18, 19, and 20 were stained. These included not only the usual resting cysts and medium-sized trophic specimens, but also young cysts, cysts in the process of excystment, and individuals just excysted. None showed a micronucleus, whereas individuals of the remaining seventeen clones, stained at the same time by the same method, invariably showed micronuclei.

The individuals of some clones (e.g., 1, 3, 5, 12) showed great diversity in micronuclear number within the clone. This fact is brought out clearly by the range which is cited for these clones, and it is further emphasized by the high standard deviations in the clones. Clone 12 showed the greatest degree of heterogeneity in that the range in micronuclear number extended from 2 to 11, with all intervening numbers being represented. On the other hand, some clones (e.g., 2, 4, 8; 13, 15, 16, 17) were relatively homogeneous, with narrow ranges and low standard deviations. Other clones lay between these extremes. Only the amicronucleate clones showed complete homogeneity.

Thus, it is seen that individuals of a clone exhibit varying numbers of micronuclei, that clones differ with respect to their mean number, and that amicronucleate clones exist in nature.

The mean number of micronuclei in the 850 micronucleate active specimens of Table I (representing 17 clones) was 7.06; the mean number in the 850 micronucleate cysts was 7.08. Unfortunately, the number in the progenitor of each clone

TABLE I

Tillina magna. *Variation in number of micronuclei in twenty clones; relation of micronuclear number to size of cysts. The clones are numbered and arranged as the mean number of micronuclei (average of means for fifty active specimens and fifty resting cysts) decreases.*

Numerical designation of clone	Range in number of micronuclei		Mean number of micronuclei ± standard deviation		Mean diameter of 50 resting cysts in microns ± standard deviation
	50 active specimens	50 resting cysts	50 active specimens	50 resting cysts	
1	10–16	9–16	12.90±1.87	12.32±1.93	85.76± 9.65
2	8–12	9–12	10.48±0.94	10.96±0.87	82.94± 7.65
3	7–14	6–13	9.62±2.30	9.24±2.08	79.68± 8.72
4	7–10	7–10	8.52±0.82	8.60±0.95	88.62± 7.25
5	6–14	6–12	8.17±1.95	8.28±1.66	88.36± 9.61
6	6–10	6–10	7.56±1.28	7.78±1.24	93.60± 6.21
7	5–10	5–10	7.25±1.28	7.38±1.36	92.44± 7.08
8	6– 9	6– 9	7.16±0.85	7.06±0.92	78.50± 7.82
9	5– 9	4– 9	6.52±1.15	6.74±1.21	84.16± 8.62
10	4– 8	4– 8	5.98±1.52	5.94±1.46	85.42± 5.28
11	5– 8	5– 9	5.90±1.03	5.76±0.96	86.14± 5.34
12	2–10	2–11	5.74±2.41	5.90±1.97	85.64± 5.32
13	4– 6	4– 6	5.12±0.42	5.10±0.45	80.64± 8.92
14	4– 8	4– 8	5.12±1.51	4.98±1.42	84.18± 4.76
15	4– 6	4– 6	4.86±0.53	5.20±0.57	81.60± 8.41
16	4– 6	4– 6	4.92±0.63	4.98±0.75	80.92± 7.37
17	3– 5	3– 5	4.28±0.75	4.14±0.74	91.42± 6.87
18	—	—	0	0	86.28± 5.63
19	—	—	0	0	84.32±10.36
20	—	—	0	0	81.52± 5.34

is unknown, since the micronuclei cannot be identified in living specimens. It seems reasonable to assume that the progenitor of each had a number approximately equivalent to the mean which was determined for the clone, and that it produced some offspring having fewer, and some having more, than its own number.

It is well known that the number of micronuclei is variable in many species of ciliates. Thus, *Paramecium multimicronucleatum* has 2 to 7 (Powers and Mitchell, 1910), though usually 4 (Wenrich, 1928); *Spathidium spathula*, 6 to 9 (Maupas, 1888, p. 247); *Urostyla grandis,* 10 to more than 40 (Tittler, 1935); and *Stentor coeruleus,* 10 to 42 within a single clone (Schwartz, 1935). On the whole, such variations within a species appear to have little effect on the structure or behavior of the individuals and to be without functional significance. This general conclusion is supported by the observations on *T. magna* which follow immediately.

NUMBER OF MICRONUCLEI IN RELATION TO VARIOUS ASPECTS OF CYSTMENT

All the clones produced normal resting cysts upon the depletion of the food supply in small stock cultures prepared with surplus animals from the lines. Furthermore, all the specimens in such cultures encysted; none persisted in prolonged swimming, thereby to perish of starvation. Hence, it is clear that the ability to encyst is not dependent on the presence of the micronucleus, since amicronucleate as well as micronucleate clones were able to encyst. Moreover, the cysts of all the clones remained viable for many months. They could be activated at any time after the fourth day by immersion in distilled water or 0.05 per cent lettuce infusion. From 2 to 2.5 hours were required for emergence at 23° C., and practically 100 per cent of the specimens excysted. No precise figures are given here, since the percentage of excystment under various conditions has been dealt with in a previous paper (Beers, 1945) and the present study contributes nothing new on this point. However, the present results show clearly that the viability of resting cysts and their capacity to excyst are in no wise related to the presence of a micronucleus, or to the number of micronuclei. Well over 90 per cent of the cysts produced in the various clones were single ones; double cysts appeared sporadically, some in amicronucleate clones, some in micronucleate. Amicronucleate cysts undergo the usual colpodid type of macronuclear reorganization, involving the extrusion of a portion of the macronuclear substance into the cytoplasm (Taylor and Garnjobst, 1941; Burt, Kidder and Claff, 1941; Beers, 1946).

The size of the cysts in different clones was made the subject of special study, for it was thought that the number of micronuclei might affect the size of the cysts. The diameters of fifty living single cysts of each clone were measured, each measurement extending from the outer surface of the ectocyst of one side to the corresponding surface of the other. The results of these measurements are included in Table I. An inspection of the table shows at once that the calculation of coefficients of correlation between micronuclear number and cyst size would be of little value, since cyst size is independent of micronuclear number. For example, if we consider certain extremes in micronuclear number, it is seen that the cysts of clone 1 had a mean diameter of about 85 μ, and those of clone 19 a diameter of 84 μ, with approximately equivalent standard deviations. Clones 2 and 20 and clones 4 and 18 constitute other examples of extreme disparity in micronuclear number with close agreement in cyst size. Among the micronucleate clones, other examples of wide divergence in micronuclear number, yet with general uniformity in cyst size, are furnished by clones 3 and 16 and by clones 6 and 17. On the other hand, some clones having widely divergent micronuclear numbers produced cysts of dissimilar sizes, e.g., clones 3 and 17 and clones 6 and 13. Thus it is seen that clones having widely different micronuclear numbers may produce cysts of equivalent mean sizes or of dissimilar mean sizes. It must be concluded that there is no relation between the number of micronuclei and the size of the cysts.

The same conclusion is reached if we adopt another approach and consider cyst size in clones which had similar, or only slightly different, micronuclear numbers. For example, clones 6 and 7 had similar mean numbers of micronuclei and they produced cysts of equivalent mean sizes; clones 15 and 16 constitute a second example. On the other hand, clones 7 and 8 had similar micronuclear numbers, but they produced cysts which differed significantly in mean size; clones 16 and 17 furnish another example. Hence, clones having similar mean micronuclear numbers may produce cysts of equivalent mean sizes or of different mean sizes. Again, it is evident that there is no relation between number of micronuclei and size of cysts. The mean diameter of the 850 micronucleate cysts of Table I was 85.23 μ; that of the 150 amicronucleate cysts was 84.04 μ.

A word concerning the size of individual cysts may be of interest. In any particular clone of *T. magna,* whether micronucleate or amicronucleate, there is usually wide variation in cyst size, even though the cysts under immediate consideration are all produced in the same small stock culture—meaning a Columbia culture dish containing 1 cc. of fluid. Cysts in such a culture often vary in size from 75 μ to 95 μ; extremes of 64 μ and 104 μ have been noted. The factors which affect cyst size appear to be of a complex physiological nature and are therefore not readily identifiable.

The point of greatest interest in this consideration of various aspects of cystment is the fact that amicronucleate and micronucleate clones behaved alike; clearly the micronucleus of *T. magna* plays a negligible role, if any, with reference to encystment, viability of cysts, excystment, macronuclear reorganization within the cysts, and cyst size.

NUMBER OF MICRONUCLEI IN RELATION TO DIVISION RATE AND VITALITY

The cultural histories of the twenty clones are presented in Table II. Clones 1, 2, 3, and 5 could not be maintained in culture for the arbitrary period of 60 days. The remaining clones were maintained with undiminished vigor and were discontinued at the end of the period.

First, the division rates of the sixteen vigorous clones will be considered in relation to micronuclear number. Clones 4, 6, 7, and 8, as has been seen, had relatively high micronuclear numbers (means, about 7 to 8.5). The total average number of generations produced by the four sub-lines of these respective clones varied from 149 to 174. Thus the clones themselves varied with respect to mean division rate. Clones 9–12 had intermediate micronuclear numbers (means, 6 to 6.5); these clones produced from 149 to 167 generations. Clones 13–17 had low micronuclear numbers (means, 4 to 5); they produced from 160 to 176 generations. Thus clones having intermediate or low micronuclear numbers produced in general as many generations, and had therefore the same division rates, as clones having relatively high micronuclear numbers.

Amicronucleate clones 18–20 produced from 154 to 172 generations. Thus the amicronucleate clones had approximately the same division rates as the micronucleate clones; e.g., clone 19 produced almost as many generations as clone 4; clone 18 produced more than clone 10; clone 20 produced about as many as clone 9 or 13. Many kinds of comparisons are possible, and the reader may choose to make other comparisons between amicronucleate and micronucleate clones. For example, clones 18–20 had higher division rates (i.e., produced more generations) than clones 8, 10, and 14; but clones 18–20 had lower division rates than clones 4, 7, and 16. Thus the general conclusion that amicronucleate clones have the same division rates as micronucleate clones is not invalidated. Sections of amicronucleate division cysts showed that the macronucleus undergoes the usual reorganization after each of its divisions, as in normal micronucleate cysts (Burt, Kidder and Claff, 1941; Beers, 1946).

Next, the vitality of the sixteen vigorous clones must be considered. An examination of the number of divisions produced in the successive 5-day periods in any particular clone shows that the clone was dividing as rapidly at the end of the experiment as at the beginning. Within the time limits of the experiment, the clones showed no decrease in vitality as measured by the division rate. How long the sixteen clones would have continued without diminution in vitality is a question that cannot be answered on the basis of the available data. The important findings are these: Some clones which have relatively high micronuclear numbers are as vigorous as those which have low numbers; amicronucleate clones are fully as vigorous as many micronucleate clones.

Clones 1, 2, 3, and 5 must receive special consideration. As has been said, these clones could not be maintained in culture for the duration of the 60-day experimental period. Clone 1 showed a rapid decrease in fission rate and encysted on the fourth day. Some of the cysts were activated and new lines were established. These in turn declined shortly and encysted. Three additional attempts were made to culture clone 1; each time the lines encysted after 3–5 days. Indeed, clone 1 was so refractory that without these repetitions it would have been impossible to obtain sufficient specimens for the usual number of micronuclear counts. Clones 2, 3, and 5 likewise could not be maintained in culture for 60 days. Their histories are presented in sufficient detail in Table II. Following the encystment of the original lines of these clones, new lines were established with excysted specimens, but they also declined and encysted after 3–4 weeks of culture. It may be maintained that the decline and encystment of these four clones resulted from a failure to meet their cultural needs. However, the conditions of culture were adequate for a total of sixteen clones, and it seems not unreasonable to assume that they were likewise adequate for clones 1, 2, 3, and 5 and to conclude that these clones declined as a result of intrinsic factors.

It has been shown that clones 1, 2, and 3 had higher micronuclear numbers than any of the other clones. Clone 5 likewise had a high number, though slightly lower than clone 4, which was cultivable. The evidence indicates that a large number of micronuclei may be detrimental to the welfare of the organism and incompatible with high vitality, but the number of such clones studied was too small to justify a general conclusion. On the whole, the results show that in *T. magna* the rate of division and the vitality of the race are in no wise related to the number of micro-

TABLE II

Tillina magna. *Relation of number of micronuclei to division rate and vitality in twenty clones maintained in pure-line culture, usually for sixty days. Four clones having high micronuclear numbers encysted before the expiration of sixty days; the remaining sixteen continued with undiminished vigor, though their mean micronuclear numbers (see Table I) varied from 8.56 to 0.*

Successive five-day periods	Total average number of divisions per line for successive periods																			
	Cl. 1	Cl. 2	Cl. 3	Cl. 4	Cl. 5	Cl. 6	Cl. 7	Cl. 8	Cl. 9	Cl. 10	Cl. 11	Cl. 12	Cl. 13	Cl. 14	Cl. 15	Cl. 16	Cl. 17	Cl. 18	Cl. 19	Cl. 20
1	5.5*	13.5	11.5	14.5	15.5	12.5	16.0	11.5	12.5	11.5	13.0	13.0	14.5	14.5	14.5	14.0	12.5	11.5	12.5	13.5
2		11.0	9.5	16.5	12.5	13.0	15.0	12.5	11.5	10.5	14.0	14.5	15.0	13.0	12.5	14.0	11.5	12.5	13.5	12.5
3		9.5	12.5	13.5	11.5	14.5	12.5	12.5	13.0	12.5	12.5	15.5	13.5	12.5	13.0	15.0	12.5	16.5	14.5	11.5
4		7.5*	10.0	14.0	10.0	11.5	14.0	13.0	12.0	15.5	11.5	13.5	14.5	11.5	16.5	15.5	13.5	12.5	15.0	14.5
5			8.5	13.0	7.0	15.0	12.5	11.5	14.5	14.5	14.0	12.5	15.0	14.5	14.0	14.5	14.5	11.0	15.5	12.5
6			9.5	16.5	0.5*	12.5	14.5	11.5	15.0	13.5	13.5	13.5	12.5	12.5	15.5	16.5	13.5	13.0	16.5	14.5
7			7.8	15.0		14.0	15.0	12.5	15.5	12.5	12.5	14.5	14.5	13.5	13.5	14.5	14.0	13.5	14.5	15.5
8			2.2*	13.5		13.5	16.5	13.5	14.0	11.5	14.5	15.0	13.5	14.5	12.5	13.0	15.0	14.5	13.0	14.5
9				14.5		13.0	13.5	11.5	16.0	12.5	13.0	13.5	11.5	11.5	14.5	14.5	14.5	12.5	14.5	13.5
10				14.0		16.0	14.0	12.5	13.5	10.5	15.0	12.5	12.5	14.0	16.0	16.5	13.5	11.0	13.5	12.5
11				15.5		12.5	15.5	13.0	12.5	13.5	12.5	14.5	14.5	12.5	13.5	14.5	13.0	12.5	15.0	13.5
12				14.0		14.0	14.5	13.5	12.0	10.5	12.5	15.0	15.0	13.0	14.5	13.5	12.5	13.0	14.0	15.5
Total number of generations	5.5	41.5	71.5	174.5	57.0	162.0	173.5	149.0	162.0	149.0	158.5	167.5	166.5	157.5	170.5	176.0	160.5	154.0	172.0	164.0

*Cl. 1. Encysted after 4 days.
*Cl. 2. Encysted after 20 days.
*Cl. 3. Encysted after 37 days.
*Cl. 5. Encysted after 26 days.

nuclei, or even to the presence of a micronucleus, since amicronucleate clones proved to be as vigorous as micronucleate clones.

The origin of the amicronucleate races cannot be accounted for with certainty. It is agreed that such races may arise at any of four periods in the life of ciliates generally: at endomixis, by the transformation of all the reconstruction micronuclei into macronuclei; at autogamy or conjugation, by an analogous transformation of all the derivatives of the synkaryon or amphinucleus into macronuclei; or at division, by an unequal distribution of the micronuclei to the daughter cells. By studying dividing individuals of an unusual race of *Paramecium caudatum*, Wichterman (1946) observed the simultaneous production of bimicronucleate and amicronucleate daughters. In *T. magna* conjugation is of rare occurrence, and endomixis and autogamy are unknown. Therefore, it is likely that amicronucleate races usually take their origin in an unequal distribution of the products of division. However, it should not be concluded from the present results that 15 per cent of all *Tillina* clones are amicronucleate; actually such clones appear to be very exceptional. I have stained many specimens during a period of 8 years; all these specimens had micronuclei, except for the members of clones 18–20.

Discussion

The functional significance of the nuclear dimorphism of the Euciliata has long held the attention of protozoologists. In general, the dimorphic condition has been viewed as representing a segregation of idiochromatin and trophochromatin, the former in the micronucleus, the latter in the macronucleus. It was originally assumed, since ciliates normally possess both types of nuclei, that both are necessary for the continued survival of the individual. Although the precise functions of the respective nuclei are difficult to determine, two lines of investigation have supplied pertinent findings, namely, a study of the capabilities of amicronucleate ciliates of natural occurrence and a study of regenerative capacity, survival, and reproduction in ciliates which have been deprived experimentally of either nucleus, or of both, whether by merotomy or other operative procedures.

The existence of naturally occurring amicronucleate races has long been conceded by such authorities as Woodruff (1921), Calkins (1930), and Reichenow (1929, p. 29) and is now accepted as a fact. The potentialities of these races, as revealed by intensive laboratory study, have demonstrated that the micronucleus is not at all necessary for the maintenance of the essential vital processes of the individual, whereas the macronucleus is indispensable. Aside from the absence of a micronucleus and the manifest inability to carry to completion such processes as endomixis, autogamy and conjugation, amicronucleate races of many ciliates do not differ structurally or physiologically from micronucleate ones.

Thus, Dawson (1919; 1920) maintained an amicronucleate race of *Oxytricha hymenostoma* in pure-line culture for 289 generations (4 months) and in small mass cultures for 5 months longer. The absence of micronuclei did not prevent the animals from attempting to conjugate, but these attempts were abortive. Woodruff (1921) cultured amicronucleate races of *Oxytricha fallax* and *Urostyla grandis* for 246 and 128 generations, respectively, and maintained a race of *Paramecium caudatum* in pure-line culture long enough to determine that it was definitely amicronucleate. A few pairs of conjugants occurred in mass cultures of *O. fallax,* but they failed to live when isolated.

Amicronucleate races of other ciliates have likewise been isolated and cultured long enough to demonstrate not only their viability but their sustained vigor and good health. Among these are the following: (1) *Spathidium spathula.* Moody (1912) was unable to find micronuclei in her specimens, though she was able to culture them for 218 generations. It is evident that they were amicronucleate, since the micronuclei of *Spathidium* were observed and counted by Maupas and were found regularly by Woodruff and Spencer (1922). (2) *Didinium nasutum.* Patten (1921) cultured an amicronucleate race, which was derived from an exconjugant of a normal micronucleate race, for 652 generations. Conjugation occurred in the amicronucleate race, but the exconjugants invariably died. The resting cysts were likewise inviable. It is evident that the race arose through the faulty reorganization of the exconjugant. (3) *Paramecium bursaria.* Woodruff (1931) cultured a race characterized by micronuclear instability for 7 years. Neither endomixis nor conjugation was observed. The race was originally bimicronucleate; later it was variable, exhibiting from 1 to 4 micronuclei; then for about 4 years it was unimicronucleate; finally, a derived race showed no micronucleus, although this race was apparently as healthy and vigorous as its bimicronucleate ancestors which were isolated 7 years earlier. Woodruff (p. 543) aptly points out that "whatever function the micronuclear apparatus plays, the somatic life of the animals is not obviously influenced by profound variations in volume or in distribution of micronuclear material." (4) *Urostyla grandis.* Tittler (1935) found amicronucleate individuals in stock cultures which previously contained only micronucleate specimens. They were indistinguishable externally from their micronucleate progenitors, and they flourished in mass cultures for 2 years. Their macronuclear divisions followed the usual complex pattern characteristic of the species. The race produced resting cysts, some of which could be excysted, although endomixis, which usually occurs in the precystic forms, was absent. Evidently some of the cysts were not entirely normal, since they showed a tendency to disintegrate, perhaps because of the omission of endomixis. (5) *Colpoda steini.* Piekarski (1939) studied comparatively the structure and reproduction of a micronucleate and an amicronucleate race and was able to culture the latter for approximately 6 years. Both races were equally cultivable and vigorous. They reproduced within division cysts from which four progeny regularly emerged and they produced normal resting cysts. They showed the same sequence of events in the division of the macronucleus. These events are of special interest, in that eight chromatic (Feulgen-positive) bodies appear in the macronucleus of the young division cyst. Ultimately each of the daughters receives two of them, and thus the behavior of these bodies suggests an equational distribution of chromosomes. Piekarski concludes that the absence of a micronucleus had no recognizable effect on the activities of the animals.

The present study of amicronucleate races of *Tillina magna* likewise demonstrates the adequacy of the macronucleus, not only for long-continued reproduction accompanied by sustained vigor, but also for encystment and excystment. Thus endowed with the ability to produce viable resting cysts, these races would seem to be capable of indefinite survival, even under the changeable conditions of a natural environment.

Still more remarkable, in connection with the capabilities of amicronucleate races, are the observations of Sonneborn (1940) and Kimball (1941) on mating types. In certain races of *Paramecium aurelia* Sonneborn found a small percentage of animals which, upon undergoing autogamy or conjugation, developed a new macronucleus from a fragment of the old macronucleus. Since the micronuclei commonly disappeared in clones produced by these animals and since the mating type never changed at macronuclear reorganization, Sonneborn concludes that "hereditary characters (including mating type) of clones from macronuclear regenerates cannot be directly determined by micronuclei, for they persist in the absence of micronuclei. Mating type must be determined by the macronucleus. . . ." Kimball was able to assign amicronucleate specimens of *Euplotes patella* to definite mating types, since they paired readily with individuals of known mating type. He concludes that "the micronucleus is thus unnecessary for an animal to be of a definite mating type."

With reference to the role of the nuclei in the regeneration of ciliates following merotomy, the results obtained with different species are not always in complete accord. The physical properties of the cytoplasm constitute an experimental variable; a fluid cytoplasm or a rigid pellicle may interfere with the closure of an injury and thus affect regeneration adversely. Balamuth (1940) has presented an excellent review of the extensive literature on this subject. For the present only a few representative examples of regeneration in ciliates will be considered, with special reference to the nuclear components of the merozoa (cell fragments).

It is well known that enucleate fragments of ciliates can neither regenerate nor continue to live, whereas nucleate fragments regenerate successfully and pursue normal lives. These conclusions are particularly evident in Balamuth's six-page tabular summary of the findings on regeneration in the ciliates. As a rule the macronucleus and micronucleus cannot be separated, and a nucleate fragment, as in Dembowska's work (1925) on *Stylonychia mytilus,* usually means one having both macronuclear and micronuclear material.

However, some investigators have succeeded in obtaining nucleate merozoa of the two types desirable for an experimental analysis of the role of the individual nuclei in regeneration; namely, macronucleate (without micronuclei) and micronucleate (without any part of the macronucleus). Reynolds (1932), for example, in work on an amicronucleate *Oxytricha fallax,* found that various types of macronucleate merozoa could regenerate their missing parts and resume their normal physiological activities. Schwartz, using microdissection techniques, was able to remove the entire macronucleus from *Stentor* and yet leave a number of micronuclei in the specimens. These micronucleate individuals never survived. By means of successive operations, he was also able to remove all the micronuclei from a few specimens, leaving a portion of the beaded macronucleus in place. These macronucleate individuals regenerated and could be cultured as pure lines. Thus he produced experimentally an amicronucleate race, which as regards size and division rate was not different from the normal controls. Bishop (1943), employing the ultra-centrifuge as a means of obtaining merozoa of *Oxytricha fallax,* obtained sixty-seven macronucleate fragments, all of which regenerated, and seven micronucleate fragments, none of which regenerated. Twelve of the regenerated macronucleate individuals were cultured as amicronucleate pure lines. Bishop concluded (p. 451) that "the lack of micronuclear material makes no difference in the regenerative capacity, division rate, motility or morphology of *Oxytricha fallax.*"

On the other hand, there is evidence that in some forms the micronucleus, as well as the macronucleus, is necesesary for regeneration and survival. Thus Reynolds found that both nuclei are necessary for the regeneration of merozoa of *Euplotes patella.* This observation is in accord with the results of Taylor and Farber (1924), who, by means of a micro-pipette, removed the micronucleus from fifty specimens of *E. patella,* all of which died within a few days. None produced more than four progeny. Hence, these authors conclude that "the micronucleus plays more than a purely germinal role in the life history of *Euplotes patella.*" However, the situation in *E. patella* is confused, for some of Kimball's unimicronucleate double animals produced viable amicronucleate individuals at division. Some of them survived as clones, though with a low division rate; one such amicronucleate clone survived for 341 days. The results show, according to Kimball (p. 30), "that the micronucleus is not essential for continued life in at least some clones of *Euplotes patella,* though its absence results in a marked decrease in vigor." In various species of *Uronychia* (Young, 1922), and in *Uroleptus mobilis* (Tittler, 1938), *Spathidium spathula* and *Blepharisma undulans* (Moore, 1924) both types of nuclei appear to be necessary for the complete regeneration, growth and division of merozoa.

Thus the evidence afforded by the long-continued culture of a number of naturally occurring amicronucleate races demonstrates conclusively that the macronucleus alone suffices for the maintenance of the vegetative life of the organism—meaning by vegetative life such diverse activities as locomotion, food capture, digestion, assimilation, growth, excretion, respiration, reproduction, and maintenance of cell proportions and form. On the basis of this evidence the normal role of the micronucleus in vegetative life appears to be one of relative passivity. The evidence from operative procedures shows that in many ciliates the macronucleus alone is adequate for complete regeneration, as well as for subsequent growth and division, whereas in other ciliates the micronucleus also is necessary. There is no authenticated case on record in which the micronucleus alone is adequate for the maintenance of vegetative functions or for regeneration. Balamuth's thorough survey of the literature leads him to make the following comment in his summary: "Of the dual nuclear apparatus, only the macronucleus can be shown to function in the actual regenerative process. The role of the micronucleus in this connection is as yet unclear; apparently it is more important in the viability of some forms than in others." On the whole, the evidence tends only to emphasize the importance of the macronucleus and to attest to the validity of Calkins' comment (1930, p. 161) on this organelle: "Far from being negligible it is on the contrary probably the most important element of the cell in matters of metabolism, reorganization, and continued cell life."

The tendency to underestimate the importance of the macronucleus in the life of the organism results probably from its apparent monotony of structure. Lacking chromosomes, its division is usually unspectacular. Nevertheless, its mode of origin is not an incidental phenomenon in the life of the ciliate, for both macro- and micronucleus almost invariably have a common and simultaneous origin. Usually they develop from the synkaryon of the conjugant, by divisions which appear to be equational. Again, they develop from the synkaryon of autogamy or from the endomictic micronucleus. Thus they inherit equally from a common nucleus of origin, and each receives equivalent chromatin elements, chief among which are presumably the genes. In the definitive micronucleus these elements retain the ability to organize periodically as chromosomes, and thereby to arrest the attention of the observer. Once in the definitive macronucleus, on the contrary, they never again assemble in the form of chromosomes. However, it is possible that they are distributed at macronuclear division by a mechanism which is fully as effective as the mitotic distribution of chromosomes, though less conspicuous. For example, it is not impossible that they are represented in multiplicate in the macronucleus and distributed at random throughout its substance. Thus each daughter at division would be reasonably assured of receiving representatives of every type of chromatin element. The behavior and the potencies of the macronuclear fragments of Sonneborn's unusual specimens of *Paramecium aurelia* indicate a multiplicate representation of the chromatin elements. In these specimens the forty or more macronuclear fragments grew and segregated during subsequent cell divisions, until there was only one in each cell. Thus each fragment was adequate for the regeneration of a complete macronucleus and for the continued life of the organism, even in the absence of micronuclei. Hence, Sonneborn concludes that "the normal macronucleus must contain at least forty complete and discrete genomes." A more precise mechanism for the distribution of the chromatin elements, involving, for example, a differential streaming of genetically equivalent elements toward opposite ends of a polarized macronucleus, may be postulated. Regardless of the type of mechanism involved in the distribution of the chromatin elements of the macronucleus at division, the fact remains that inheritance in an amicronucleate ciliate is no less precise, to judge by the structure and physiological activities of the offspring, than in a micronucleate ciliate. The fact that the behavior of the macronucleus does not conform to the chromosome theory of heredity in *sensu stricto,* in that chromosomes are absent, may mean simply that a different mechanism for the distribution of the genes is involved.

Whether the macronucleus of amicronucleate ciliates may justifiably be regarded as an amphinucleus—one containing idiochromatin as well as trophochromatin, as Woodruff (1921), Moore and others have suggested—seems doubtful in the light of recent investigations. Thus it has been shown by Schwartz and by Bishop that viable amicronucleate races of *Stentor* and *Oxytricha* may be derived by experimental means from normal individuals in which the idiochromatin and trophochromatin were presumably segregated in the two types of nuclei. The macronucleus of these individuals, following removal of the micronuclei, was adequate to maintain all the usual vegetative activities in the derived amicronucleate races.

In conclusion, the evidence shows that the macronucleus is the essential nuclear element in the vegetative life of ciliates. The micronucleus functions largely, if not solely, in the periodic replacement of the macronucleus and in the production of new genetic combinations, some of which undoubtedly render the species better adapted to survival. The nature of the physiological conditions which call for a renewal of the macronucleus is not clear; that such renewal meets an imperative physiological need is shown by the widespread occurrence of the phenomenon in the Euciliata.

SUMMARY

The number of micronuclei was examined in 50 trophic specimens and 50 resting cysts of each of 20 clones of *Tillina magna,* three of which were amicronucleate.

In any particular clone trophic specimens and resting cysts contained approximately equivalent mean numbers of micronuclei. In different micronucleate clones the mean number varied from 4.21 to 12.61. The mean for 1,700 specimens of 17 clones was 7.07.

The number in the individuals of any particular micronucleate clone was variable; some clones showed relatively little variation, e.g., 3 to 5 micronuclei; others, considerable variation, e.g., 2 to 11 micronuclei. The smallest number observed in any micronucleate individual was 2; the largest, 16.

All the clones produced normal resting cysts upon depletion of the food supply (*Pseudomonas fluorescens*). The cysts of different clones were equally viable and capable of excystment. Their size was unaffected by the number of micronuclei. Amicronucleate cysts showed the usual macronuclear reorganization. Hence, neither the number of micronuclei nor the absence of micronuclei affected encystment, viability and size of cysts, excystment or macronuclear reorganization.

An attempt was made to maintain each clone in pure-line culture for 60 days and thereby to examine the division rate and vitality. Four clones were refractory and encysted before 60 days expired. The remaining 16 clones, including the three amicronucleate ones, survived with undiminished vigor and were discontinued. The 13 micronucleate clones produced from 149 to 176 generations during the 60-day period; the three amicronucleate clones produced 154, 164, and 172 generations, respectively. Hence, the 16 surviving clones showed slight differences in their average daily division rates, but neither the divison rate nor the vitality of these clones was correlated with variations in micronuclear number or with the absence of micronuclei. Division cysts of amicronucleate clones showed the usual macronuclear reorganization after each division of the macronucleus. The four refractory clones had high micronuclear numbers.

Since conjugation is rare and endomixis and autogamy are unknown in *Tillina*, it is probable that amicronucleate races arise at division by an unequal distribution of the daughter micronuclei.

Some of the literature on amicronucleate ciliates and on the regeneration of various types of nucleate merozoa is reviewed. The evidence shows that the macronucleus is the indispensable nuclear element in the so-called vegetative life of the organism, whereas the micronucleus during this period appears to be a relatively passive organelle. Its chief function concerns the periodic replacement of the macronucleus and the production of new hereditary combinations. Special attention is directed to the fact that inheritance in an amicronucleate race is no less precise than in a typical micronucleate race, although the division of the macronucleus is amitotic and usually reveals no suggestion of true chromosomes. It is evident that the hereditary mechanism of amicronucleate races, and perhaps of ciliates generally, differs radically from the conventional chromosomal mechanism of metazoa.

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